Vitamin A Supplementation and Genital Shedding of Herpes Simplex Virus among HIV-1–Infected Women: A Randomized Clinical Trial

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Cross-sectional analyses have associated vitamin A deficiency with genital shedding of herpes simplex virus (HSV) among human immunodeficiency virus type 1 (HIV-1)–infected women. A randomized clinical trial of vitamin A supplementation given daily for 6 weeks was conducted among 376 women in Mombasa, Kenya, who were coinfected with HSV-2 and HIV-1. At follow-up, there was no significant difference in the detection of genital HSV DNA between women receiving vitamin A supplementation and women receiving placebo (40% vs. 44%, respectively; P = .5) Among women shedding HSV, there was no significant difference in the mean HSV DNA quantity between the group that received vitamin A supplementation and the group that received placebo (4.51 vs. 4.67 log₁₀ copies/swab; P = .6). HSV shedding was associated with significantly higher vaginal and cervical HIV-1 shedding, even after controlling for the plasma HIV-1 load and the CD4 count. Vitamin A supplementation is unlikely to decrease HSV shedding and infectivity.

Herpes simplex virus (HSV) type 2 is a common co-infection among persons with HIV-1, and studies have suggested significant synergy between the global epidemics of the 2 viruses [1]. The presence of HSV-2 increases the risk of HIV-1 acquisition [2], and it has been suggested that regional variation in the seroprevalence of HSV-2 may be one of the principal factors that explains the spread of HIV-1 in Africa [3]. HSV-2 reactivation occurs with greater frequency among HIV-1–infected individuals than among individuals not infected with HIV-1, and it may increase shedding of HIV-1 in genital tract secretions [4–6]. Thus, interventions that decrease HSV-2 reactivation may reduce transmission of both HSV-2 and HIV-1.

We previously reported the results of a cross-sectional study of genital shedding of HSV in HIV-1–infected women [7]. Among women who were not pregnant and were not using hormonal contraception, lower serum concentrations of vitamin A were strongly associated with increased detection of HSV. Randomized trials performed among vitamin A–deficient populations have demonstrated that vitamin A supplementation can be an effective intervention to reduce morbidity associated with infectious diseases [8]. With these results in mind, we conducted a randomized, double-blind, placebo-controlled trial to assess the effect of vitamin A supplementation on shedding of HSV.

METHODS

Participants and procedures. From September 1998 through June 2000, a total of 400 HIV-1–seropositive women who were attending outpatient clinics at Coast Provincial General Hospital in Mombasa, Kenya, were...
enrolled in the present study, as described elsewhere [9]. Criteria for exclusion from the study included age <18 years or >45 years, pregnancy, or use of vitamin supplements or oral contraceptive pills. The primary aim of the trial was to assess the effect of vitamin A on genital shedding of HIV-1. Therefore, enrollment in the study was delayed for women with abnormal cervical or vaginal discharge or ulceration, because these conditions increase shedding of HIV-1 [10]; such women could not be enrolled in the study until after they completed treatment, according to the guidelines of the Kenya Ministry of Health. Treatment for genital ulcer disease did not include the use of medications active against HSV. Informed consent was obtained from all study participants. The study was approved by the institutional review boards of the University of Washington (Seattle) and the University of Nairobi (Nairobi, Kenya), and the human-experimentation guidelines of these institutions were followed.

Women were interviewed by use of a standard questionnaire. Blood samples were obtained, and pelvic examination was performed (by J.M.B. or R.S.M.). For assessment of shedding of HSV, a Dacron swab was rolled across the ectocervix, vulva, perineum, and perianal area and then was placed into a dry cryovial. Samples of vaginal and cervical secretions were obtained for detection of shedding of HIV-1, as described elsewhere [6, 9].

Computer-generated block randomization was used to assign women to receive a 6-week supply of gelatin capsules that were to be taken daily and that contained either 10,000 IU of vitamin A as retinyl palmitate or placebo (Tishcon). This regimen is recommended by the World Health Organization for correction of vitamin A deficiency in women [11]. Pill bottles were coded with random numbers, and the regimens were indistinguishable. Prescription bottles that contained an alarm (RemindRx; IBV Technologies) were used to remind patients to take their daily dose. After 6 weeks, the women returned to the study clinic so that follow-up specimens could be obtained and pill counts performed.

**Laboratory methods.** HIV-1 serologic testing was done by ELISA (Detect HIV-1/2 [BioChem ImmunoSystems]; results, if positive, were confirmed by the World Recombigen [Cambridge Biotech]). Type-specific serologic testing for HSV-1 and HSV-2 was also done by ELISA (HerpeSelect; Focus). The CD4 cell counts were determined using a semiautomated system (Zym- mune; Bartels), which had a limit of detection of 25 cells/μL. Serum samples were tested for the presence of vitamin A by use of high-pressure liquid chromatography. Vitamin A deficiency was defined by concentrations <30 μg/dL [8, 9].

HSV DNA was assayed using real-time polymerase chain reaction analysis, as described elsewhere [12]. This assay uses primers to the common region of HSV glycoprotein B, and, thus, it detects both HSV-1 and HSV-2. The Gen-Probe HIV-1 load assay was used for quantification of HIV-1 RNA, with lower limits of detection of 6 copies/mL, for plasma samples, and 15 copies/swab, for cervical and vaginal swabs [13].

**Data analysis.** The trial was designed to include 200 study participants in each arm, with assessment of shedding of HIV-1 as the primary aim [9]. Determination of the effect of vitamin A supplementation on genital shedding of HSV was a secondary aim of the trial. The sample size was sufficient to detect a 3-fold reduction in shedding of HSV, as a result of vitamin A supplementation, under assumptions of 80% power, a 2-sided test for which \( \alpha = 0.05 \), a 15% loss to follow-up, a 93% seroprevalence of HSV-2, and a 17% prevalence of shedding of HSV among seropositive women, as seen in our earlier cross-sectional study [7].

The effect of vitamin A supplementation on shedding of HSV was assessed by intent-to-treat analysis. We performed a cross-sectional analysis of the association between shedding of HSV and HIV-1, using data obtained from the study participants at enrollment. The CD4 cell counts and HIV-1 RNA loads below the limit of detection were set at one-half the respective limits. Categorical variables were compared by use of chi² tests, and continuous variables were compared using independent samples t tests and Pearson’s correlation coefficients. HSV and HIV-1 loads were log₁₀ transformed to approximate normality. Multivariate linear and logistic regression models were used. For the multivariate models, CD4 cell counts were square root transformed. Analyses were conducted using SPSS (version 10.0; SPSS).

**RESULTS**

Of 400 HIV-1–infected women who were enrolled in the trial, 376 (94%) were HSV-2 seropositive; 192 of these 376 women received vitamin A, and 184 received placebo. At enrollment, characteristics of the women were comparable between the randomization groups (table 1). The study participants had relatively advanced HIV-1 disease: 44% had CD4 cell counts <200 cells/μL, and the mean plasma HIV-1 load was 5.41 log₁₀ copies/mL. Seventy-four (20%) used either depot medroxyprogesterone acetate (DMPA) or Norplant forms of hormonal contraception. Most women (91%) were seropositive for HSV-1.

After 6 weeks, the women returned to the study clinic so that follow-up specimens could be obtained and pill counts performed. Analyses were conducted using SPSS (version 10.0; SPSS).
prescribed pills. Follow-up and compliance did not differ according to randomization group (P > .2 for all comparisons). Study participants who were lost to follow-up had more advanced HIV-1 disease than did study participants who completed the study (mean CD4 cell count, 162 vs. 280 cells/µL [P < .001]; mean plasma virus load, 5.80 vs. 5.36 log10 copies/mL [P < .001]), and they were more likely to be vitamin A deficient (76% vs. 56%; P = .009); however, they were not significantly more likely to be shedding HSV (48% vs. 40%; P = .3).

**Effect of vitamin A on HSV shedding.** At follow-up, there was no statistically significant difference in the prevalence of genital shedding of HSV DNA in women randomized to receive vitamin A, compared with women randomized to receive placebo (40% vs. 44%; P = .5) (table 2). Among women who shed HSV, the mean quantity of genital HSV DNA did not differ between women who received vitamin A and women who received placebo. Genital ulceration was present in 39 women (12%) and did not differ according to randomization group (20 of 168 women given vitamin A vs. 19 of 162 women given placebo; P = 1.0).

We considered whether the high prevalence of immunosuppression among our study population limited the response to vitamin A supplementation, by performing subgroup analyses restricted to women with CD4 cell counts >200 cells/µL at enrollment. In comparisons of women who received vitamin A with women who received placebo, there was no significant difference in the prevalence of shedding of HSV at follow-up (39 [35%] of 111 women vs. 29 [34%] of 85 women, respectively; P = .9) or in the quantity of HSV detected among women who shed HSV (4.36 vs. 4.46 log10 copies/swab, respectively; P = .8). There also was no significant difference in the prevalence or quantity of HSV shedding among the subgroup of women who returned for follow-up within 8 weeks of randomization (data not shown).

In our previous cross-sectional study [7], the association of vitamin A status with shedding of HSV was strongest after the exclusion of women who were pregnant or were using hormonal contraception. Thus, we repeated our analyses among the subgroup of women who were not using DMPA or Norplant (pregnancy and use of oral contraceptive pills were trial exclusion criteria). We observed no effect of vitamin A supplementation on the prevalence or quantity of shedding of HSV. Additional subgroup analyses among women with vitamin A deficiency did not demonstrate any significant effect of supplementation on shedding of HSV, either among the entire study population or among participants who were not using DMPA or Norplant.

**Association between HSV and HIV-1 shedding.** At enrollment, women who shed HSV had higher concentrations of HIV-1 in plasma (mean, 5.49 vs. 5.35 log10 copies/mL; P = .1), vaginal secretions (mean, 3.39 vs. 2.86 log10 copies/swab; P < .001), and cervical secretions (mean, 3.44 vs. 3.10 log10 copies/swab; P = .007) than did women who did not shed HSV. After adjustment for plasma virus load and CD4 cell count,
Third, our regimen of vitamin A supplementation was chosen because it is sufficient for replenishment of vitamin A in women who have clinical signs of very severe vitamin A deficiency, such as corneal damage, which was not seen in this cohort [11]. Although it would be interesting to know whether any changes would become apparent with longer follow-up, it seems unlikely that inadequate sample size or supplementation explains our findings.

In our trial, at enrollment, we found no strong association between vitamin A deficiency and shedding of HSV, which potentially suggests either that the association seen in our earlier study was spurious or that deficiency does not increase shedding in all populations. In addition, although we used endocervical samples for measurement of shedding of HSV in our previous study [7], in the present study, we used a multisite swab that collected samples from the ectocervix, vulva, perineum, and perianal area, because these are more biologically plausible sites for HSV transmission. It is difficult to hypothesize a mechanism by which vitamin A may influence shedding of HSV from some genital sites but not from others. Thus, it seems unlikely that the different specimen-collection techniques would account for the different results in our 2 studies.

In the primary analysis from this trial [9], we found that vitamin A supplementation had no effect on shedding of HIV-1, although earlier studies had found strong associations between low serum levels of vitamin A and HIV-1 infectivity. Subsequent analysis suggested that low levels of vitamin A may reflect more active or advanced HIV-1 disease, rather than true deficiency [14], implying that observational studies of vitamin A and HIV-1 were confounded by the severity of HIV-1 disease.
Figure 1. Vaginal (A) and cervical (B)/HIV-1 RNA concentrations from the study enrollment visit, according to increasing quantities of herpes simplex virus (HSV) DNA. Squares and whiskers denote the mean and 95% confidence interval of the mean, respectively, of the HIV-1 RNA concentration (log{10} copies/swab) in vaginal and cervical samples, as categorized by HSV DNA quantity. Mean HIV-1 RNA concentration is also shown for each HSV DNA category. The largest subgroup evaluated (n = 223) includes women who had no HSV DNA detected; 1 woman from this group had undergone a hysterectomy and, thus, contributed only a vaginal sample for HIV-1 RNA analysis. Women who shed HSV were categorized into quartiles, according to HSV DNA quantity. The mean ± SD for each quartile was as follows: quartile 1 (2.32 ± 0.29 log_{10} copies/swab), quartile 2 (3.87 ± 0.44 log_{10} copies/swab), quartile 3 (5.27 ± 0.52 log_{10} copies/swab), and quartile 4 (6.97 ± 0.58 log_{10} copies/swab). There were significant correlations between the HSV DNA quantity and the vaginal and cervical HIV-1 load (Pearson’s correlation coefficient \( r = 0.21, P < .001 \) and \( r = 0.18, P = .001 \), respectively). After adjustment for plasma HIV-1 load and CD4 cell count, by use of multivariate linear regression analyses, HSV quantity remained significantly associated with the HIV-1 RNA concentration in both vaginal and cervical secretions (\( P = .003 \) and \( P = .03 \), respectively).
It seems likely that our previous study of vitamin A levels and shedding of HSV, which found deficiency to be associated with a >10-fold increase in detection of HSV among women who were not pregnant or were not using hormonal contraception, may have been similarly confounded [7].

One limitation of the present study is that a single follow-up sample may be inadequate to reflect the day-to-day pattern of shedding of HSV, because HSV is intermittently detectable in the genital tract [15]. Detailed evaluations of shedding of HSV, including daily sampling, may be more ideal for assessment of the magnitude of effect that interventions may have on detection of HSV. Nonetheless, our results provide no evidence that vitamin A supplementation reduces HSV reactivation.

Two previous studies found a significant correlation between the quantities of HSV DNA and HIV-1 RNA in genital tract secretions of coinfected women, although this correlation was found only in subgroup analyses that were restricted to women who were shedding HSV [5, 6]. The results of the present study demonstrate that the quantity of HIV-1 in the genital tract is significantly higher among women who shed any amount of HSV than among those who do not shed HSV, and they confirm a strong association between the quantities of HIV-1 and HSV that are shed. These findings strengthen the argument that HSV reactivation increases HIV-1 infectivity. Few women in the present study had genital ulcer disease, and the associations of HSV with shedding of HIV-1 were unchanged after women with ulcers were excluded from the study, emphasizing that the effect of HSV on HIV-1 infectivity may be largely a result of subclinical HSV reactivation.

In summary, the present trial suggests that vitamin A supplementation is unlikely to reduce shedding or infectivity of HSV. The results emphasize the importance of randomized clinical trials to verify or refute associations discovered in cross-sectional studies. The present study confirms that shedding of HSV is common among women coinfected with HSV-2 and HIV-1 and that it may increase HIV-1 infectivity, even in the absence of genital ulceration. Interventions to decrease HSV reactivation, such as HSV suppressive therapy with acyclovir [15], should be pursued as potential strategies to decrease shedding and transmission of HIV-1.

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References